ATP-independent binding in heart. Furthermore, in heart and muscle we could explain the decrease in the $Kpu_{in\text{-}vivo}$ values by in-vitro binding studies, while in liver this was not so.

REFERENCES

Ball, W. J., Tse-Eng, D., Wallick, E. T., Bilezikan, J. P., Schneider, B., Butler Jr, V. P. (1981) J. Clin Invest. 68: 1065–1074

Chen, H. G., Gross, J. F. (1979) J. Pharmacokinet. Biopharm. 7: 117–125

Doering, W. (1979) N. Engl. J. Med. 301: 400–404

Eaton, D. L., Klaassen, C. D. (1978) J. Pharmacol. Exp. Ther. 205: 480–488

Ejvinsson, G. (1978) Br. Med. J. 1: 279–280

Geiger, J. D., Das, G., Parmar, S. S. (1982) Pharmacol. 25: 177–182

Gelbart, A., Goldman, R. H. (1977) Biochim. Biophys. Acta. 481: 689–694

Gibson, T. P., Nelson, H. A. (1980) J. Lab. Clin. Med. 95: 417–428

Gold, H., Modell, W., Price, L. (1932) Arch. Int. Med. 50: 766–796

Harashima, H., Sugiyama, Y., Sawada, Y., Iga, T., Hanano, M. (1984) J. Pharm. Pharmacol. 36: 340–342

Harrison, L. I., Gibaldi, M. (1976) Drug Metabol. Disposit. 4: 88–93

Kim, D. H., Akera, T., Brody, T. M. (1981) J. Pharmacol. Exp. Ther. 217: 559–565

Kuschinsky, K., Lullmann, H., van Zwiten, P. A. (1968) Br. J. Pharmacol. Chemother. 32: 598–608

Leahey, E. B., Reiffel, J. A., Drusin, R. E., Heissenbuttel, R. H., Lovejoy, W. P., Bigger, J. T. (1978) J. Am. Med. Ass. 240: 533–534

Lin, J. H., Sugiyama, Y., Awazu, S., Hanano, M. (1982) J. Pharmacokinet. Biopharm. 10: 637–647

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Sato, J., Sawada, Y., Iga, T., Hanano, M. (1983) J. Pharm. Sci. 72: 1137–1141

Schenck-Gustafsson, K., Dahlqvist, R. (1981) Br. J. Clin. Pharmacol. 11: 181–186

Straub, K. D., Kane, J. J., Bissett, J. K., Doherty, J. E. (1978) Circulation 58, suppl. 2, II–58

Mechanisms of the relaxations induced by 5-hydroxytryptamine on the rat isolated caecum

MARY O. UGURU*, S. O. A. BAMGBOSE, *Department of Pharmacology, College of Medicine, University of Lagos, P.M.B. 12003, Lagos, Nigeria*

The mechanism by which 5-HT produces relaxations of the rat caecum has been examined. Propranolol and cocaine markedly attenuated the relaxations whereas tetrodotoxin had no effect. Higher doses of propranolol and cocaine ($>10^{-6}$ M), as well as reserpinization, converted the relaxations into contractions. There was a residual relaxation resistant to propranolol, cocaine and reserpine treatment. High doses of 5-HT ($>10^{-5}$ M) were thus thought to relax the rat caecum indirectly, through the release of noradrenaline from the tissue by a tyramine-like action. The relaxations do not seem to be due only to the release of noradrenaline.

The resting tone of the rat caecum is such that the preparation responds to application of a drug with contractions or relaxations depending on the drug used. Sympathomimetic drugs cause relaxation and cholinomimetics (bradykinin and prostaglandins E_1, E_2 and F) contraction. Low doses of 5-hydroxytryptamine ($<10^{-6}$ M) produce a contraction which is converted to relaxation in the presence of atropine (Tayo & Acholem 1981). Higher doses of 5-HT ($>10^{-6}$ M) produce relaxation of the caecum in the absence of any antagonist (Uguru 1983). The possibility that this relaxation might be due to the release of noradrenaline from the tissue has been investigated. Adrenergic axons in other tissues are known to be capable of taking up 5-HT and

* Correspondence and present address: Department of Pharmacology, Faculty of Medical Sciences, Unviersity of Jos, PMB2084, Jos, Nigeria.

releasing noradrenaline (Innes 1962; Owman 1964; Pluchino 1972).

Materials and methods

Albino rats, 200–350 g, were killed and exsanguinated. The intestine was exposed and the caecum cut at the ileo-caecal junction. The appendix was removed and the caecal contents flushed out with Tyrode solution of the composition (mM); NaCl 137, KCl 2·7, $MgCl_2$ 0·5, $CaCl_2$ 1·36, NaH_2PO_4 0·3, $NaHCO_3$ 11·9, glucose, 11·1. The tissue, mounted in a 50 ml organ bath containing Tyrode solution between 35–37 °C and bubbled with air, was left for 1 h to equilibrate, the Tyrode solution being replaced every 15 min. Responses were recorded on an Ugo Basile Gemini recorder (CAT 7070) by means of an Ugo Basile isotonic transducer (CAT 7006). The tension on the tissue was between 0·8–1 g.

5-HT was added non-cumulatively to the bath and was left for 1 min before washing. 2–3 min were allowed between applications of 5-HT. Increasing concentrations of propranolol, cocaine or tetrodotoxin were added cumulatively to the Tyrode solution in the reservoir and tissues were exposed to each concentration for at least 30 min before addition of 5-HT. Other rats were treated with reserpine 4 mg kg^{-1}, 18–20 h before the addition of 5-HT.
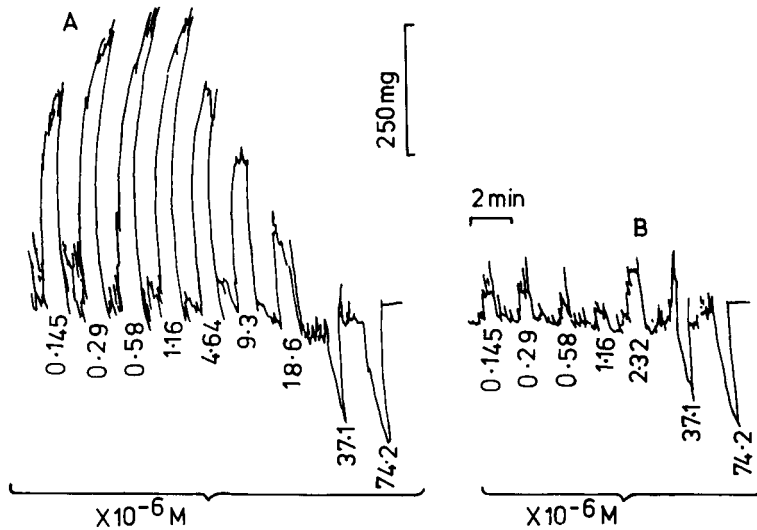
FIG. 1. A. Typical response of the rat isolated caecum induced by 5-HT. Vertical scale: tension of isolated tissue in mg. Horizontal calibration: time in min. B. The development of tachyphylaxis to the contractile effect which did not affect the relaxations. The responses were obtained 5 min after A.

Drugs used. These were: 5-hydroxytryptamine creatinine sulphate (Sigma), propranolol (ICI), cocaine (May & Baker), reserpine (Ciba) and tetrodotoxin (Sankyo).

Stock solutions of the drugs were made in distilled water and these were diluted with Tyrode solution for the experiment. 5-HT was dissolved with a few drops of concentrated hydrochloric acid before being made up to volume with distilled water. The concentrations of the drugs are expressed as the final organ bath concentration in moles.

Results

Concentration–response curve for 5-HT on the rat isolated caecum. Low doses of 5-HT ($3 \cdot 6 \times 10^{-8} - 5 \cdot 8 \times 10^{-7}$ M) caused dose-dependent contractions of the caecum. These reached a plateau, after which the

contractions gradually declined. Higher doses of 5-HT ($1 \cdot 9 \times 10^{-5} - 7 \cdot 5 \times 10^{-5}$ M) produced concentration-dependent relaxations (Fig. 1A).

Tachyphylaxis to the contractile effect of 5-HT. After the initial dose-response curve was completed, subsequent contractions to 5-HT were markedly reduced (Fig. 1B). The tachyphylaxis persisted after washing the preparation at 15 min intervals for 1 h. However, the relaxations were reproducible and started to decline only after 4–5 dose-response curves were obtained.

Effect of propranolol and cocaine on the relaxations. Propranolol ($3 \cdot 0 \times 10^{-8} - 3 \cdot 0 \times 10^{-6}$ M) and cocaine ($5 \cdot 7 \times 10^{-7} - 5 \cdot 7 \times 10^{-5}$ M) caused a dose dependent inhibition of the relaxations induced by 5-HT (Figs 2, 3).
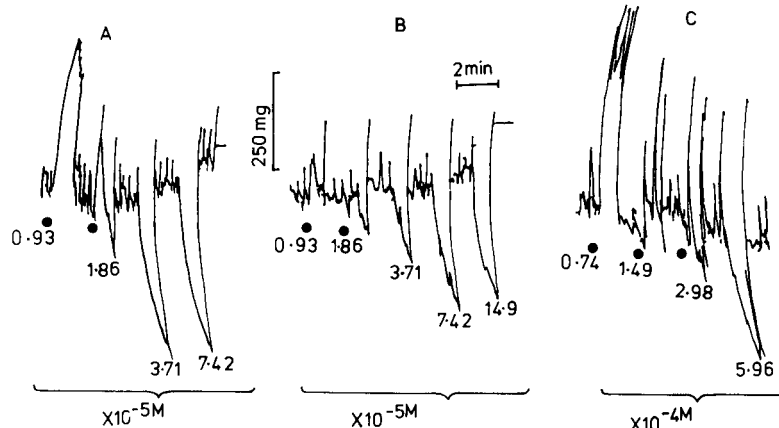

FIG. 2. Effect of increasing concentrations of propranolol on the relaxations induced by 5-HT. A = control, B and C are responses in the presence of propranolol 3×10^{-8} and 3×10^{-6} M respectively. The black dots indicate addition of 5-HT. Vertical scale: tension of isolated tissue in mg. Horizontal calibration: time in min.
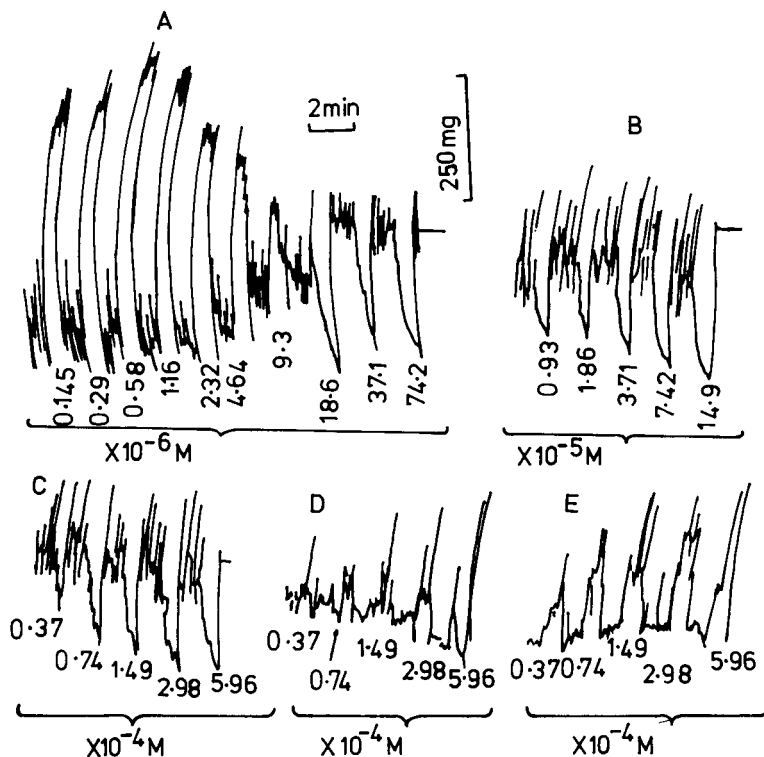
FIG. 3. A typical effect of cocaine on the relaxations induced by 5-HT. A = control, B = responses obtained 5 min after A, C, D and E = responses in the presence of increasing concentrations of cocaine 5.7×10^{-7}, 5.7×10^{-6} and 5.7×10^{-5} M respectively. Vertical scale: tension of isolated tissue in mg. Horizontal calibration: time in min.
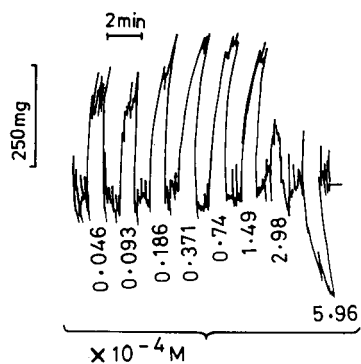

FIG. 4. Typical responses to 5-HT in preparations from rats pretreated with reserpine, 4 mg kg^{-1}, 18–20 h before the experiment. Vertical scale: tension of isolated tissue in mg. Horizontal calibration: time in min.

Higher doses of these drugs ($>10^{-6}$ M) converted the relaxations into contractions. There was a residual relaxation, even in the presence of high doses of both drugs.

Effect of reserpine pretreatment. Concentrations of 5-HT, which caused relaxations in control tissues, produced contractions in tissues from rats pretreated with reserpine (cf. Figs 1A, 4). Rats treated with

reserpine showed less tachyphylaxis to the contractile effect of 5-HT and up to four dose-response curves were obtained without tachyphylaxis being seen.

Effect of tetrodotoxin. Tetrodotoxin (10^{-7} – 10^{-5} M) did not have any effect on the relaxations (Fig. 5A, B). The decrease seen in the height of the contractions in Fig. 5B may be due to tachyphylaxis as observed in the control experiments (e.g. Fig. 1B).

Discussion
Low doses of 5-HT produced contractions of the caecum while higher doses produced relaxations. Tachyphylaxis developed to the contractile effect of 5-HT on this tissue in a manner similar to other parts of the rat intestine (Gillan & Pollock 1980; Farmer & Laniyonu 1984). The relaxations were reproducible and might be due to the action of noradrenaline released from the tissue by 5-HT as they were similar to those induced by exogenously applied noradrenaline (Uguru 1983) although the doses were much higher in the case of 5-HT. Noradrenaline (10^{-9} – 10^{-8} M) always produced dose-dependent relaxations of the tissue. Propranolol, which blocks the action of noradrenaline on the tissue, also antagonized the relaxations induced by 5-HT. Cocaine, which blocks the uptake mechanism of drugs into adrenergic axons, and reserpine, which
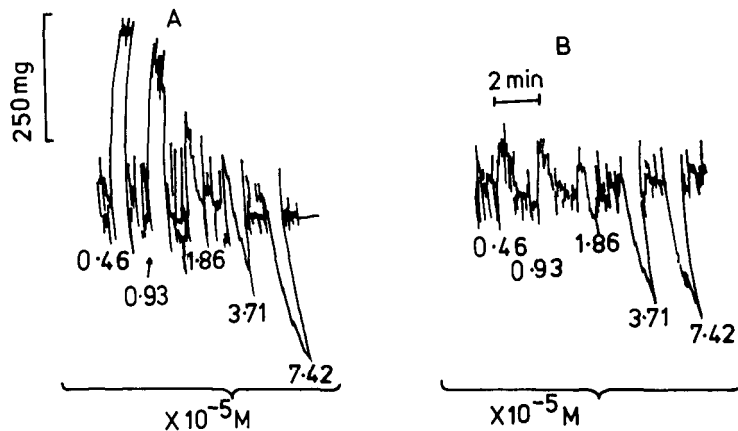
FIG. 5. Effect of tetrodotoxin $3 \cdot 1 \times 10^{-6}$ M on the responses of the rat caecum induced by 5-HT. A = control, B = responses in the presence of tetrodotoxin. Vertical scale: tension of isolated tissue in mg. Horizontal scale: time in min.

depletes adrenergic nerves of noradrenaline, converted the relaxations into contractions.

The relaxations were not affected by tetrodotoxin, suggesting that 5-HT could displace noradrenaline by a mechanism which did not involve action potentials. Tetrodotoxin blocks sodium channels without affecting the store of transmitters in the nerve endings (Oriowo 1981) and it is possible that 5-HT could be releasing noradrenaline from these stores by a tyramine-like action which is not blocked by tetrodotoxin, but is antagonized by cocaine (Smith 1973). This ability of adrenergic axons to take up 5-HT and release noradrenaline by a tyramine-like action has been widely reported (Innes 1962; Owman 1964; Shasken & Snyder 1970). The residual relaxations in the presence of high concentrations of cocaine and propranolol and after rats had been pretreated with reserpine may be due to the stimulation of inhibitory tryptamine receptors as reported by Bucknell & Whitney (1964) and Burleigh (1977) for the human colon.

In conclusion, adrenergic nerves in the rat caecum are capable of taking up 5-HT and releasing noradrenaline. It is possible that at least part of the tachyphylaxis to the contractile effect of 5-HT on the tissue is due to a physiological antagonism by noradrenaline. This is supported by the observation that the tachyphylaxis did not occur so readily in tissues from reserpine pretreated rats. These results suggest that to investigate the contractile effect of 5-HT on the rat caecum, it is necessary to destroy the adrenergic nerves or to deplete the tissue of its noradrenaline content.

REFERENCES

Bucknell, A., Whitney, B. (1964) Br. J. Pharmacol. 23: 164–175

Burleigh, D. E. (1977) J. Pharm. Pharmacol. 29: 538–541

Farmer, J. B., Laniyonu, A. A. (1984) Br. J. Pharmacol. 82: 883–889

Gillan, M. G. C., Pollock, D. (1980) Ibid. 68: 381–392

Innes, I. R. (1962) Br. J. Pharmacol. Chemother. 19: 427–441

Oriowo, M. A. (1981) Arch. Int. Pharmacodyn. 254: 13–19

Owman, C. (1964) Int. J. Neuropharmacol. 3: 105–112

Pluchino, S. (1972) Naunyn-Schmiedeberg's Arch. Pharmacol. 272: 189

Shasken, E. G., Snyder, S. M. (1970) J. Pharmacol. Exp. Ther. 175: 404–418

Smith, A. D. (1973) Br. Med. Bull. 29(2): 123–129

Tayo, F. M., Acholem, M. O. (1981) Arch. Int. Pharmacodyn. 254: 20–27

Uguru, M. O. (1983) M.Sc. Thesis. University of Lagos